NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 405

TOXICOLOGY AND CARCINOGENESIS STUDIES OF C.I. ACID RED 114 (CAS NO. 6459-94-5) IN F344/N RATS (DRINKING WATER STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF C.I. ACID RED 114

(CAS NO. 6459-94-5)

IN F344/N RATS

(DRINKING WATER STUDIES)

A desalted commercial dye containing approximately 85% 1,3-Naphthalenedisulfonic acid, 8-((3,3′-dimethyl-4′-((4-(((4-methylphenyl)sulfonyl)oxy)phenyl)azo)(1,1′-biphenyl)-4-yl)azo)-7-hydroxy-disodium salt, 10% structurally related compounds, 1%-4% water, and 1% sodium chloride

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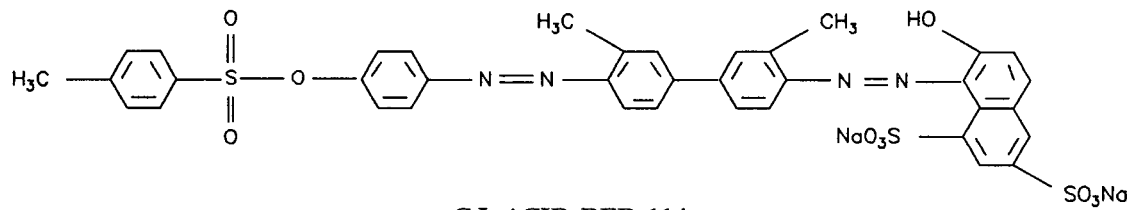
ABSTRACT



C.I. ACID RED 114

CAS No. 6459-94-5

Chemical Formula: $C_{37}H_{28}N_4O_{10}S_3Na_2$ Molecular Weight: 830.8

Synonyms: 1,3-Naphthalenedisulfonic acid, 8-((3,3′-dimethyl-4′-((4-(((4-methylphenyl)sulfonyl)oxy)phenyl)azo)(1,1′-biphenyl)-4-yl)azo)-7-hydroxy, disodium salt, Acid Leather Red BG, Acid Red 114, Amacid Milling Red PRS, Benzyl Fast Red BG, Benzyl Red BR, Cerven Kysela, C.I. 23635, Erionyl Red RS, Folan Red B, Kayanol Milling Red RS, Leather Fast Red B, Levanol Red GG, Midlon Red PRS, Milling Red B, Milling Red BB, Milling Red SWB, NCI C61096, Polar Red RS, Sandolan Red N-RS, Sella Fast Red RS, Sulphonol Fast Red R, Supranol Fast Red GG, Supranol Red PBX-CF, Supranol Red R, Telon Fast Red GG, Tertracid Milling Red B, Vondamol Fast Red RS

C.I. Acid Red 114 is one of five chemicals being evaluated in 2-year carcinogenicity and toxicity studies as part of the NTP's Benzidine Dye Initiative. This Initiative was designed to evaluate representative benzidine congeners, benzidine congener-derived dyes, and benzidine-derived dyes. C.I. Acid Red 114 was nominated for study because of the potential for human exposure during production of bisazobiphenyl dyes and because benzidine, a structurally related chemical, is a known human carcinogen.

Toxicology and carcinogenesis studies were conducted by administering desalted, industrial grade C.I. Acid Red 114 in drinking water to groups of F344/N rats of each sex for 13 days, 13 weeks, 9 or 15 months, or 2 years. These studies were performed only in rats because studies of benzidine congeners were being performed in mice at the National Center for Toxicological Research (NCTR). Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary cells, and *Drosophila melanogaster*.

13-Day Studies

Rats were exposed to C.I. Acid Red 114 in drinking water at doses of 0, 10,000, 20,000, or 30,000 ppm. All control and dosed rats survived except one male rat in the 20,000 ppm dose group. Final mean body weights in the three dosed groups were 94%, 83%, or 77% of controls for males and 92%, 88%, or 80% of controls for females. Water consumption declined with increased dose. Clinical findings included red stained fur, ears, and tail in all test animals. On gross necropsy, organs and tissues were also stained red.

13-Week Studies

C.I. Acid Red 114 was administered in drinking water at doses of 0, 600, 1,200, 2,500, 5,000, or 10,000 ppm. All control and dosed animals survived until the end of the study. Final mean body weights in the five dosed groups were 97%, 89%, 87%, 87%, or 85% of controls for males and 97%, 94%, 94%, 92%, or 89% of controls for females. Water consumption was decreased in dosed animals. As was seen in the 13-day studies, major organs and tissues from treated animals were stained red. Kidney toxicity characterized by regeneration and karyomegaly of tubule epithelial cells with chronic inflammation was observed in female rats at doses of 1,200 ppm or above. Treatment-related increases in relative liver weights and elevated liver enzyme levels were seen in males and females,

centrilobular pallor in the liver was seen in all male dose groups. Because of these body weight differences, decreases in water consumption, and organ toxicity, the doses chosen for the 2-year studies were 70, 150, and 300 ppm for males and 150, 300, and 600 for females.

2-Year Studies

Male rats received doses of 0, 70, 150, or 300 ppm of C.I. Acid Red 114, and female rats received 0, 150, 300, or 600 ppm. Seventy animals were in the control and high-dose groups, 45 in the low-dose groups, and 75 in the mid-dose groups. Ten animals were evaluated from the control and high-dose groups at 9 months, and ten animals from all dose groups were evaluated at 15 months. The average amount of compound consumed per day was 4, 8, or 20 mg/kg for males and 9, 20, or 70 mg/kg for females.

Survival and Body Weights

Survival at 105 weeks for male rats receiving 0, 70, 150, or 300 ppm was 24/50, 15/35, 26/65, and 1/50; for females receiving 0, 150, or 300 ppm, survival was 36/50, 13/35, and 6/64. All female rats receiving 600 ppm died by week 89. The decreased survival in treated groups was due primarily to the development of chemical-related neoplasms. Of the surviving animals, the final mean body weights for males receiving 70 or 150 ppm were 94% and 90% of control and for females receiving 150 or 300 ppm, 99% and 84% of control. These weight differences began in the second year of the studies and were attributed in part to the development of neoplasms in the dosed groups.

Histopathologic Effects in the 2-Year Studies

At 9 and 15 months, a few neoplasms were seen in the liver, lung, clitoral gland, skin, Zymbal's gland, oral cavity epithelium, and small and large intestine, and the number of neoplasms at these sites increased as the studies progressed. At 2 years, there was a clear carcinogenic response in the skin, Zymbal's gland, and liver of male and female rats, and in the clitoral gland, oral cavity epithelium, small and large intestine, and lung in female rats. Treatment-related increases were also seen in the incidence in neoplasms of the oral cavity epithelium, adrenal gland, and lung of male rats, and in mononuclear cell leukemia and in neoplasms of the mammary gland and adrenal gland in female rats. The incidence of these neoplasms was generally lower, but was significant and considered to be marginally related to chemical treatment. The same neoplastic effects have been previously observed in some or all of the NTP studies with dimethoxybenzidine, dimethylbenzidine, or C.I. Direct Blue 15.

Genetic Toxicology

In a standard preincubation protocol, C.I. Acid Red 114 was mutagenic in *Salmonella typhimurium* strain TA98 in the presence of induced hamster liver S9, and an equivocal response was noted in strain TA100 with hamster liver S9. However, no significant mutagenic activity was noted in strains TA1535 or TA1537 with or without S9 activation. In a modified *S. typhimurium* gene mutation test which employed reductive metabolism followed by oxidative metabolism with S9 liver enzymes, C.I. Acid Red 114 was strongly mutagenic in strain TA1538. C.I. Acid Red 114 did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells with or without S9 activation; reductive metabolism was not used in these cytogenetic tests. No increase in sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* administered C.I. Acid Red 114 by feeding or injection.

Conclusions

Under the conditions of these 2-year drinking water studies, there was *clear evidence of carcinogenic activity** of C.I. Acid Red 114 for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, and liver. Increased incidences of neoplasms of the oral cavity epithelium, adrenal gland, and lung may have been related to chemical administration. There was *clear evidence of carcinogenic activity* for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity epithelium, small and large intestines, and lung. Increased incidences of mononuclear cell leukemia, mammary gland adenocarcinoma, and adrenal gland pheochromocytomas may have been related to chemical administration.

*Explanation of Levels of Evidence of Carcinogenic Activity appears on page 8. A summary of peer review comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of C.I. Acid Red 114

Male F344/N Rats	Female F344/N Rats
Drinking water concentration	
0, 70, 150, or 300 ppm C.I. Acid Red 114	0, 150, 300, or 600 ppm C.I. Acid Red 114
Body weights	
Dosed were 9% lower than controls during second year	Dosed were 24% lower than controls during second year
2-Year survival rates[a]	
24/50, 15/35, 26/65, 1/50	36/50, 13/35, 6/64, 0/50
Nonneoplastic effects	
None	None
Neoplastic effects[b]	
Skin basal cell neoplasms: 1/50, 5/35, 28/65, 32/50	Skin basal cell neoplasms: 0/50, 4/35, 7/65, 5/50
Skin keratoacanthoma: 1/50, 1/35, 4/65, 7/50	Zymbal's gland neoplasms: 0/50, 3/35, 18/65, 19/50
Skin sebaceous cell neoplasms: 1/50, 1/35, 5/65, 6/50	Clitoral gland neoplasms: 11/48, 17/32, 28/62, 23/50
Skin squamous cell neoplasms: 1/50, 2/35, 11/65, 9/50	Liver neoplasms: 0/50, 0/35, 19/64, 8/50
Zymbal's gland neoplasms: 0/50, 0/35, 8/65, 7/50	Lung neoplasms: 1/50, 2/35, 9/65, 4/50
Liver neoplasms: 2/50, 2/35, 15/65, 20/50	Oral cavity epithelium neoplasms: 0/50, 3/35, 9/65, 6/50
	Small intestine neoplasms: 0/50, 0/35, 1/65, 2/50
	Large intestine neoplasms: 0/50, 1/35, 0/65, 3/50
Uncertain findings	
Oral cavity epithelium neoplasms: 0/50, 0/35, 1/65, 2/50	Mammary gland adenocarcinoma: 0/50, 3/35, 6/65, 3/50
Adrenal gland pheochromocytomas: 17/50, 11/35, 27/63, 21/49	Adrenal gland pheochromocytomas: 1/50, 3/35, 5/64, 1/50
Lung neoplasms: 2/50, 2/35, 2/65, 3/50	Mononuclear cell leukemia: 12/50, 13/35, 18/65, 5/30
Level of evidence of carcinogenic activity	
Clear evidence	Clear evidence
Genetic toxicology	
Salmonella typhimurium gene mutation:	Positive with S9 in strain TA98; equivocal with S9 in strain TA100; Negative with or without S9 in strain TA1535 or TA1537
Salmonella typhimurium with reductive metabolism:	Positive in strain TA1538
Sister chromatid exchange in Chinese hamster ovary cells *in vitro*:	Negative with or without S9
Chromosomal aberration in Chinese hamster ovary cells *in vitro*:	Negative with or without S9
Drosophila melanogaster germ cell mutation:	Negative by feeding or injection

[a] Reduced survival in exposed groups was due to neoplasia.
[b] Number with lesion/total evaluated

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence)** and **(some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that because of major flaws cannot be evaluated **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.

- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.

- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.

- **Inadequate study** of carcinogenic activity describes studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- the adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- the presence or absence of dose relationships;
- the statistical significance of the observed tumor increase;
- the concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the NTP draft Technical Report on C.I. Acid Red 114 on March 11, 1991 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenicity activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS

On March 11, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of C.I. Acid Red 114 received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Committee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J. K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of C.I. Acid Red 114 by noting this was one of five chemicals being evaluated as part of the NTP Benzidine Dye Initiative, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplasms and nonneoplastic lesions in male and female rats. The proposed conclusions were *clear evidence of carcinogenic activity* for male and female F344/N rats.

Dr. Zeise, a principal reviewer, agreed in principle with the conclusions. However, she proposed that increased incidences of mononuclear cell leukemia and thyroid follicular cell neoplasms in female rats may have been related to chemical administration and should be cited as such in the conclusions.

Dr. McKnight, the second principal reviewer, agreed with the conclusions. She also thought that the increased incidences of mononuclear cell leukemia and thyroid follicular cell neoplasms in female rats may have been related to chemical administration. In response to Drs. Zeise and McKnight, Dr. Dunnick stated that there was not enough evidence to support even a marginal finding for thyroid tumors in that there was an increase only in the low-dose group, no increase by the trend test, and no increase in precursor hyperplastic lesions in dosed groups. With regard to mononuclear cell leukemia in female rats, Dr. Dunnick commented that incidences in dosed groups were within the historical control range and that high and early mortality in the high-dose group was felt to be due to toxicity of the chemical and not mononuclear cell leukemia. Dr. McKnight pointed out that by the life table test, the test normally used for mononuclear cell leukemia, the pairwise comparison of each of the dose groups with the control group is statistically significant, and the trend test is highly significant.

Dr. Davis, the third principal reviewer, agreed with the conclusions. He asked why the doses in female rats were double those in males since hematologic data and data on kidney degeneration from the 13-week studies suggested females were more sensitive to toxic effects. Dr. Dunnick said apparent liver toxicity in males in the 13-week studies was the primary reason for the different dose levels used in 2-year studies.

Dr. Zeise moved that the Technical Report on C.I. Acid Red 114 be accepted with the revisions discussed and the conclusions as written for male and female rats, *clear evidence of carcinogenic activity*, with the addition of mononuclear cell leukemia to the conclusion for female rats as "may have been related to chemical administration." Dr. McKnight seconded the motion, which was accepted unanimously with ten votes.